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TITLE: Targeting Estrogen-Induced COX-2 Activity in Lymphangioleiomyomatosis (LAM)

PRINCIPAL INVESTIGATOR: Jane Yu

CONTRACTING ORGANIZATION:

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13. SUPPLEMENTARY NOTES

Lymphangioleiomyomatosis (LAM), prostaglandin biosynthesis, cyclooxygenase-2 (COX-2), COX-2 inhibitors, xenograft tumors, bioluminescent imaging, cell proliferation

14. ABSTRACT:

Lymphangioleiomyomatosis (LAM) is a progressive neoplastic disorder that leads to lung destruction and respiratory failure primarily in women. LAM is typically due to TSC2 mutations resulting in mTORC1 activation in proliferative smooth muscle-like cells in the lung. The female predominance of LAM suggests that estradiol contributes to disease development. Metabolomic profiling identified an estradiol-enhanced prostaglandin biosynthesis signature in Tsc2-deficient cells, both in vitro and in vivo. Estradiol increased the expression of cyclooxygenase-2 (COX-2), a rate-limiting enzyme in prostaglandin biosynthesis, which was also increased at baseline in TSC2-deficient cells, and was not affected by rapamycin treatment. However both Torin 1 treatment and Rictor knockdown, led to reduced COX-2 expression and phospho-Akt-S473. Prostaglandin production was also increased in TSC2-deficient cells. In preclinical models, both Celecoxib and aspirin reduced tumor development. LAM patients had significantly higher serum prostaglandin levels than healthy women. 15-epi-lipoxin-A4 was identified in exhaled breath condensate from LAM subjects and was increased by aspirin treatment, indicative of functional COX-2 expression in the LAM airway. In vitro, 15-epi-lipoxin-A4 reduced the proliferation of LAM patient-derived cells in a dose-dependent manner. Targeting COX-2 and prostaglandin pathways may have therapeutic value in LAM and TSC-related diseases, and possibly in other conditions associated with mTOR-hyperactivation.

15. SUBJECT TERMS : none listed

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1. INTRODUCTION:

This proposal is focused on the molecular mechanisms underlying the pathogenesis of lymphangioleiomyomatosis (LAM), a devastating pulmonary disease affecting exclusively young women, often leading to end-stage lung disease. LAM is believed to affect approximately 30% of women with tuberous sclerosis complex (TSC). The only proven treatment for LAM is lung transplantation, which carries significant one-year mortality and after which LAM can recur in the transplanted lungs. The pathogenesis of LAM is very unusual: LAM cells are histological benign smooth muscle cells carrying TSC1 or TSC2 mutations that are believed to metastasize to the lungs where they cause lung degeneration. Cells lacking TSC1 or TSC2 exhibit hyperactivation of the mammalian target of rapamycin complex 1 (mTORC1), a master regulator of cell growth, protein translation, and metabolism. In LAM patients the mTORC1 inhibitor Rapamycin stabilizes lung function and improves symptoms. Our central hypothesis is that E_2 induces COX-2 activity and production of prostaglandins (PGE_2, PGD_2, and 6-K-$PGF_{1\alpha}$), thereby promoting the survival and lung metastasis of TSC2-null cells. Furthermore, in preclinical models of LAM, molecular and pharmacologic suppression of COX-2 will block the E_2-promoted lung metastasis and induce a regression of established lung lesions.

2. KEYWORDS:

Lymphangioleiomyomatosis (LAM), prostaglandin biosynthesis, cyclooxygenase-2 (COX-2), COX-2 inhibitors, xenograft tumors, bioluminescent imaging, cell proliferation

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Aim 1. To examine the cellular impact of COX-2 in TSC2-null LAM patient-derived cells in vitro. We proposed to test whether COX-2 is a key mediator for E_2-enhanced prostaglandin production. We have successfully established two independent clones of COX-2 shRNA in human lung epithelial cells (Figure 1). We will use the same set of shRNA-COX-2 to develop COX-2 knock-down LAM patient-derived cells. We have also developed the ELISA assay for measuring PGE_2 and 6-K-$PGF_{1\alpha}$ in conditioned media.

Aim 2. To determine whether the molecular depletion of COX-2 suppresses estrogen-promoted lung metastasis of LAM patient-derived cells in vivo. We have successfully established two independent clones of COX-2 shRNA in TSC2-null LAM patient-derived cells. We will perform the in vivo experiment in the second funding year. To measure COX-2 activity in tumors of TSC2-null cells, we used noninvasive live imaging-XenoLight RediJect COX-2 probe. However, we did not detect tumor specific COX-2 signals in mice bearing xenograft tumors. We will continue optimizing the reagent and monitor tumor COX-2 activity.

Aim 3. To determine whether the COX-2 inhibitor Celecoxib can reduce the burden of established tumors or block E_2-promoted lung metastases in preclinical models of LAM

What was accomplished under these goals?

1). COX-2 is a key mediator for E_2-enhanced prostaglandin production. To inhibit COX-2 pharmacologically, we treated TSC2-deficient cells with aspirin or NS398, and found that both agents reduced COX-2 protein levels and production of PGE_2 and 6-K-$PGF_{1\alpha}$. This result has been published (Figure 4j-k, Li et al., J Expt Med 2014, please see the Appendix 1).

2) Inhibition of COX-2 inhibits growth rate of TSC2-null cells. We found, aspirin or NS398, COX-2 inhibitors, suppressed the proliferation of TSC2-deficient cells. This result has been published (Figure 4l, Li et al., **J Exp Med** 2014, please see the Appendix 1).

3) Inhibition of COX-2 suppresses the growth of subcutaneous tumors. We have treated mice with the COX-2 inhibitor aspirin, and measured xenograft tumor burden in a subcutaneous tumor model of TSC2-null cells. We found that aspirin treatment for three weeks decreased the intensity of bioluminescence,

and decreased the tumor size. Tumors also had reduced expression of COX-2 and c-Myc, and increased levels of cleaved-caspase-3 and cleaved-PARP. This result has been published (Figure 5c-f, Li et al., **J Exp Med** 2014, please see the Appendix 1).

What opportunities for training and professional development has the project provided?

This project generated preliminary and published data that have been used for receiving two awards:

1. Chenggang Li, PhD, was awarded a three-year Postdoctoral Fellowship-the LAM Foundation (2014/1 – 2017/12)
2. Jane Yu, PhD, was awarded a three-year RO1-NIH/NIDDK

How were the results disseminated to communities of interest?

1. Oral presentation at The International LAMposium 2014. April, 2014 Chicago IL.
   "Excessive prostaglandin biosynthesis exacerbate tumor development in LAM."
2. Poster presentation at AACR-The Translational Impact of Model Organisms on Cancer 2013, November, 2013 San Diego, CA.
   "Aspirin inhibits COX-2-mediated prostaglandin production and tumorigenesis in TSC."
3. Manuscript published J Exp Med 2014.
   "Estradiol and mTORC2 enhance prostaglandin biosynthesis and tumorigenesis in tuberous sclerosis complex."
4. Manuscript published PLOS One 2014.
   "Rapamycin-insensitive up-regulation of adipocyte phospholipase A2 in tuberous sclerosis complex and lymphangioleiomyomatosis."

What do you plan to do during the next reporting period to accomplish the goals?

Nothing to Report.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

LAM is often a progressive disease which leads to respiratory failure and death in the absence of lung transplantation. The recent demonstration that rapamycin has clinical benefit in LAM is a major success. However, not all patients respond to rapamycin, and upon rapamycin withdrawal, lung function decline resumes. Hence lifelong treatment of LAM patients with rapamycin may be required to maintain benefit, with unknown long-term toxicities.

Our findings suggest that aspirin and/or other COX-1/COX-2 inhibitors may have significant benefit in slowing LAM progression. The well-known side-effect and toxicity profile of these drugs make them attractive candidates for long-term therapy in LAM patients.

What was the impact on other disciplines?

It is also possible that other neoplastic conditions associated with mTOR hyperactivation could be responsive to these agents. Further preclinical and clinical investigation is warranted to explore these possibilities.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

We demonstrated that COX-1/COX-2 inhibition may have significant benefit in slowing LAM progression and make it promising for long-term therapy in LAM patients.

5. CHANGES/PROBLEMS:

Actual or anticipated problems or delays and actions or plans to resolve them

To measure COX-2 activity in tumors of TSC2-null cells, we used noninvasive live imaging-XenoLight RediJect COX-2 probe. However, we did not detect tumor specific COX-2 signals in mice bearing xenograft tumors. We will continue optimizing the reagent and monitor tumor COX-2 activity.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

1). Chenggang Li, Po-Shun Lee, Yang Sun, Xiaoxiao Gu, Erik Zhang, Yanan Guo, Chin-Lee Wu, Neil Auricchio, Carmen Priolo, Jing Li, Alfredo Csibi, Andrey Parkhitko, Tasha Morrison, Anna Planaguma, Shamsah Kazani, Elliot Israel, Kai-Feng Xu, Elizabeth Petri Henske, John Blenis, Bruce Levy, David Kwiatkowski, Jane Yu. Estradiol and mTORC2 cooperate to enhance prostaglandin biosynthesis and tumorigenesis in tuberous sclerosis complex. The Journal of Experimental Medicine (JEM). 2014 Jan 13;211(1):15-28 PMID: 24395886, PMCID: PMC3892971 (published) Acknowledgement of federal support (yes).

2) Li C, Zhang E, Sun Y, Lee P, Zhan Y, Guo Y, Osorio JC, Rosas IO, Xu K, Kwiatkowski D and **Yu JJ**. (2014) Rapamycin-insensitive up-regulation of adipocyte phospholipase A2 in tuberous sclerosis complex and lymphangioleiomyomatosis. **PLOS One.** 2014 (In Press). Acknowledgement of federal support (yes).

Other publications, conference papers, and presentations.

1) Oral presentation at The International LAMposium 2014. April, 2014 Chicago IL.
"Excessive prostaglandin biosynthesis exacerbate tumor development in LAM."
2) Poster presentation at AACR-The Translational Impact of Model Organisms on Cancer 2013, November, 2013 San Diego, CA.
"Aspirin inhibits COX-2-mediated prostaglandin production and tumorigenesis in TSC."

- **Inventions, patent applications, and/or licenses**

Treatment of Lymphangioleiomyomatosis, January 1,2014. non-provisional, application number: 043214077221.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

1) Name:	John Blenis
Project Role:	Collaborator
Researcher Identifier (e.g. ORCID ID):	n.a.
Nearest person month worked:	less than 1
Contribution to Project:	Dr. Blenis has suggested and reviewed manuscript in the area of mTORC2 regulated COX-2 activity.
Funding Support:	National Institutes of Health Grant,GM51405
2) Name:	Bruce D. Levy
Project Role:	Collaborator
Researcher Identifier (e.g. ORCID ID):	n.a.

Nearest person month worked:	less than 1
Contribution to Project:	Dr. Levy has provided, analysis, and reviewed area of 15-epi-LXA_4 profiles in LAM patients.
Funding Support:	National Heart Lung and Blood Institute grants, HL68669
3) Name:	Elizabeth P. Henske
Project Role:	Collaborator
Researcher Identifier (e.g. ORCID ID):	n.a.
Nearest person month worked:	less than 1
Contribution to Project:	Dr. Henske has provided, analysis, and reviewed area of metabolites profile signature of prostaglandins in ELT3 cells, xenograft tumors and in LAM patients.
Funding Support:	The LAM Foundation, The Adler Foundation,The LAM Treatment Alliance, and National Heart Lung and Blood Institute grants HL118760
4) Name:	David Kwiatkowski
Project Role:	Collaborator
Researcher Identifier (e.g. ORCID ID):	n.a.
Nearest person month worked:	less than 1
Contribution to Project:	Dr. Kwiatkowski has provided, analysis, and reviewed area of expression profile of prostaglandins in patient-derived cells, and in vivo study of effect of celecoxib on kidney cystadenoma in heterozygous mice.
Funding Support:	National Cancer Institute grants, 1P01CA120964

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report.

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

1) Organization Name: Massachusetts General Hospital

Location of Organization: Boston

Partner's contribution to the project (identify one or more)

- Collaboration (e.g., partner's staff work with project staff on the project);

2) Organization Name: Peking Union Medical College

Location of Organization: Beijing, China

Partner's contribution to the project (identify one or more)

- Collaboration (e.g., partner's staff work with project staff on the project);

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) shall be updated and submitted with attachments.

9. APPENDICES:

1) Chenggang Li*, Po-Shun Lee*, Yang Sun*, Xiaoxiao Gu, Erik Zhang, Yanan Guo, Chin-Lee Wu, Neil Auricchio, Carmen Priolo, Jing Li, Alfredo Csibi, Andery Parkhitko, Tasha Morrison, Anna Planaguma, Shamsah Kazani, Elliot Israel, Kai-Feng Xu, Elizabeth Petri Henske, John Blenis, Bruce D Levy, David Kwiatkowski and **Jane J. Yu**. Estradiol and mTORC2 orchestrate to enhance prostaglandin biosynthesis and tumorigenesis in tuberous sclerosis complex. **J Exp Med 2014.**
2) Li C, Zhang E, Sun Y, Lee P, Zhan Y, Guo Y, Osorio JC, Rosas IO, Xu K, Kwiatkowski D and **Yu JJ**. (2014) Rapamycin-insensitive up-regulation of adipocyte phospholipase A2 in tuberous sclerosis complex and lymphangioleiomyomatosis. **PLOS One.** 2014 (In Press)

SUPPORTING DATA:

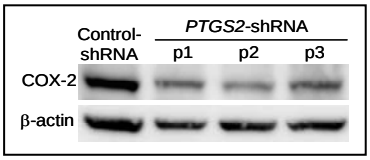

Figure 1. Development of stable cells in which COX-2 is depleted. Human bronchial epithelial BEAS-2A cells were infected with lentiviral shRNA against *PTGS2* (COX-2) and grown with puromycin 3 μg/ml for 1 week. After selection the cells were grown with puromycin 1 μg/ml. COX-2 levels were examined at passages p1-p3.